BRONCHODILATING ACTIONS OF (±)-1-(3,4,5-TRIMETHOXYS
BENZYL)-1,2,3,4-TETRAHYDROISOQUINOLINE (TMI)
DERIVATIVES IN ANESTHETIZED CATS

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Accepted April 6, 1977

Abstract—Bronchodilating actions of 7-hydroxy(I), 5,7-dihydroxy(II), 5,6,7-trihydroxy
(III) and 6,7,8-trihydroxy(IV)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroiso
quinoline (TMI) were investigated in anesthetized cats and results were compared to
those of AQ-110 (6,7-dihydroxy-TMI) and isoproterenol. TMI derivatives given
intravenously suppressed the bronchoconstriction induced by serotonin. These
compounds also caused an increase in heart rate and a decrease in diastolic pressure.
All of these actions were inhibited by pretreatment with propranolol, suggesting that
they were classified as β-sympathomimetics. Bronchodilating activities of AQ-110,
I, II, III and IV were 1.26, 1.36, 1.40, 1.77 and 1.23 that of isoproterenol, respectively.
When compared with isoproterenol, I and the other TMI derivatives were about 9 and
2 times more potent in producing bronchodilation than in increasing heart rate. On
the other hand, the bronchodilating activities of I, II and AQ-110 were 2-3 times as
great as their depressor activities, while III and IV had no selectivity. When admini-
stered into the duodenum, II was the most potent in producing bronchodilation and
the potency was followed by those of AQ-110, I, III, isoproterenol and IV in decreasing
order. Duration of the bronchodilating action of TMI derivatives administered via
either intravenous or intraduodenal route was considerably longer than that of iso-
proterenol; especially I and II were long-acting. These results suggest that I and II exhibit
a greater absorption efficiency or intraduodenal bioavailability than the other com-
ponds tested.

As to the bronchodilating action of tetrahydroisoquinolines, Iwasawa and Kiyomoto
(1) reported that the chemical structure essential for full manifestation of the activity should
include 1,2,3,4-tetrahydroisoquinoline nucleus, two free hydroxy groups at position 6 and
7, and arylmethyl group at position 1. They also demonstrated that 3,4,5-trimethoxybenzyl
analogue was the most active among arylmethyl groups tested. The contribution of hydroxy
groups to the bronchodilating action of 1,2,3,4-tetrahydroisoquinoline derivatives possessing
3,4,5-trimethoxybenzyl group at position 1, however, remained unclear.

The present experiments were performed to investigate the bronchodilating activities
of 7-hydroxy, 5,7-dihydroxy, 5,6,7-trihydroxy and 6,7,8-trihydroxy groups of (±)-1-
(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (TMI) in anesthetized cats. To
determine β-adrenoceptor selectivities of these compounds, effects on the cardiovascular
system were also studied. Isoproterenol (Iso) and 6,7-dihydroxy-TMI (AQ-110 (1-8))
were included for comparative purposes.
Cats of either sex, weighing 1.8 to 4.2 kg, were anesthetized with sodium pentobarbital (40 mg/kg) given intraperitoneally. Artificial ventilation was carried out with a constant volume respiratory pump (Acorna, AR-300) through a tracheal cannula. The ventilation rate was 25 to 30 strokes/min and the stroke volume was 13 to 15 ml/kg body weight. Animals were then immobilized with gallamine triethiodide 8 to 10 mg/kg, i.v. Intratracheal pressure, the increase of which denotes the bronchoconstriction, was measured by means of a pressure transducer (Nihon Kohden, LPU-0.1) connected to the side arm of the tracheal cannula. Bronchoconstriction was produced by injection of serotonin creatinine sulfate (5-HT), 20 \( \mu g/kg \) i.v. After these responses to 5-HT became consistent, the test compound dissolved in saline was administered into the cannulated femoral vein or into the duodenum through a catheter that terminated with a 22 gauge needle inserted into the duodenum. The bronchodilating activity of the compound was estimated by measuring the percent decrease in intratracheal pressure increased by 5-HT. In experiments with intravenous injections, Iso was given 1 min prior to the administration of 5-HT, while the TMI derivative was 2 min prior to 5-HT as it has a slower onset of action. 5-HT was injected at 10 min intervals and experiments were continued until the intratracheal pressure recovered to the initial level. When the test compound was administered into the duodenum, doses which produced approx. 70\% reduction of bronchoconstriction were chosen. Cats were challenged with 5-HT at a given interval 5 min after intraduodenal administration of the test compound.

Blood pressure was recorded from the femoral artery by means of a pressure transducer (Nihon Kohden, MPU-0.5). Heart rate was measured by cardiotachograph, triggered by arterial pulses. All responses were recorded simultaneously on a polygraph (Nihon Kohden, RM-85).

Geometric mean ED values with 95\% confidence intervals (C.I.) (9) were calculated for the TMI derivatives and compared to the calculated ED value for Iso to obtain the relative

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**FIG. 1.** Chemical structure of (±)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (TMI) derivatives.
activities and activity ratios. Parallelism between dose-response curves was analyzed by the parallel line assay (10).

Drugs used in the present experiments were: sodium pentobarbital (Tanabe Seiyaku), gallamine triethiodide (Teikoku Kagakusangyo), serotonin creatinine sulfate (Tokyo Kasei), isoproterenol hydrochloride (Boehringer Sohn), propranolol hydrochloride (I.C.I.). The TMI derivatives were synthesized in our laboratory. Chemical structures are shown in Fig. 1.

RESULTS

Intravenous studies

Effects on 5-HT induced bronchoconstriction: Intravenous injection of Iso and TMI derivatives reduced the bronchoconstriction induced by 5-HT. Fig. 2-A illustrates dose-response curves for peak inhibition by Iso and the TMI derivatives of the 5-HT-induced bronchoconstriction. As shown in Fig. 2-A, dose-response curves for bronchodilation of the TMI derivatives were parallel to that of Iso, except for I. The curves for I at higher doses was less steep than for Iso. When activities were compared at doses which caused
50% inhibition (ED₅₀), Iso was the most potent and the potency was followed by these of AQ-110, II, III, IV and I in decreasing order. Of the five TMI derivatives, AQ-110 exhibited the greatest potency, while the activity of I was weakest: the potencies of AQ-110 and I were 1/2.6 and 1/36 that of Iso, respectively.

It was also evident that the bronchodilating actions of the TMI derivatives continued for considerably longer than that of Iso (Fig. 3). Among the TMI derivatives, I and II produced longer-lasting effects than AQ-110, III and IV. Under the condition in which the same peak response (approx. 75% of control) was elicited, the half-duration (see the legend in Table 2) was approx. 40 min for I and II, 20 min for AQ-110, III and IV, and less than 10 min for Iso.

**Effects on heart rate:** Fig. 2-B represents dose-response curves for peak increases in heart rate obtained for Iso and the TMI derivatives. Similar to the effects on bronchodilation, the dose-response curves of AQ-110, II, III, IV and Iso were in parallel, but the curve for I was obviously less steep than that for Iso (P<0.01). As shown in Fig. 2-B, the positive chronotropic action of Iso was the strongest, followed by AQ-110, II, III, IV and I in that decreasing order. At doses which were necessary for increasing the heart rate by 15 beats/min (HR₁₅), the potencies of AQ-110 and I were 1/4.9 and 1/350 that of Iso, respectively. Time course for the positive chronotropic action of each compound was similar to that for the bronchodilating effect described above.

**Effects on diastolic pressure:** When injected into the femoral vein, Iso and the TMI derivatives reduced the diastolic pressure in anesthetized cats. Dose-response curves for depressor actions of these compounds are shown in Fig. 2-C. Values represented in the figure were those of the response at the peak time. As illustrated in Fig. 2-C, all the compounds tested gave parallel dose-response curves and the activities of Iso, AQ-110, II, III, IV and I decreased in this order. As estimated by the dose for fall of the diastolic pressure by 25 mmHg (DP₂₅), the potency of AQ-110 was 1/4.5 that of Iso, whereas I was 1/110 as potent as Iso. The effects of Iso and the TMI derivatives on the diastolic pressure lasted

![Fig. 4](image-url). Effects of isoproterenol ( Iso) and the TMI derivatives, given intravenously, on blood pressure, heart rate and the 5-HT-induced bronchoconstriction before and after treatment with propranolol in the anesthetized cat. B.P.: blood pressure, H.R.: heart rate, I.T.P.: intratracheal pressure. ●: 5-HT 20 μg/kg, i.v.
<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Bronchodilation</th>
<th>Heart rate</th>
<th>Diastolic Pressure</th>
<th>Activity</th>
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<tr>
<td></td>
<td></td>
<td>ED&lt;sub&gt;95&lt;/sub&gt;</td>
<td>A Relative Activity (Iso=100)</td>
<td>HR&lt;sub&gt;15&lt;/sub&gt;</td>
</tr>
<tr>
<td>Iso</td>
<td>23</td>
<td>0.033</td>
<td>(0.023-0.049)</td>
<td>0.013</td>
</tr>
<tr>
<td>AQ-110 6,7-OH</td>
<td>10</td>
<td>0.087</td>
<td>(0.064-0.12)</td>
<td>0.064</td>
</tr>
<tr>
<td>I 7-OH</td>
<td>5</td>
<td>1.2</td>
<td>(0.47-3.0)</td>
<td>4.5</td>
</tr>
<tr>
<td>II 5,7-OH</td>
<td>7</td>
<td>0.13</td>
<td>(0.074-0.23)</td>
<td>0.11</td>
</tr>
<tr>
<td>III 5,6,7-OH</td>
<td>6</td>
<td>0.26</td>
<td>(0.16-0.44)</td>
<td>0.22</td>
</tr>
<tr>
<td>IV 6,7,8-OH</td>
<td>6</td>
<td>0.76</td>
<td>(0.42-1.4)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

a) ED values were calculated in micrograms per kilogram with 95% C.I. for 50% inhibition of the 5-HT-induced bronchoconstriction (ED<sub>95</sub> %) and for absolute changes in heart rate (HR<sub>15</sub> beats/min) and diastolic pressure (DP<sub>25</sub> mmHg).
for a short period and their actions almost disappeared within 10 min after intravenous injection.

Effects of propranolol: Fig. 4 represents the effect of propranolol on the bronchodilating, positive chronotropic and depressor actions of Iso and the TMI derivatives. These actions were conspicuously inhibited by pretreatment with propranolol (0.5 mg/kg i.v.).

ED values and activity ratios: Table 1 summarizes the ED values and activity ratios of Iso and the TMI derivatives in regard to inhibition of the 5-HT-induced bronchoconstriction, increase in heart rate and decrease in diastolic pressure in anesthetized cats. Activity ratios of the TMI derivatives, i.e., the ratios of the bronchodilating activity to the positive chronotropic or depressor activity, were expressed as values relative to the activity ratio for Iso. It was demonstrated in Table 1 that the TMI derivatives exerted their actions more selectively on the bronchial system than on heart rate when compared with Iso, and I was the most selective. The activity ratios of I was approx. 9, whereas those of the other TMI derivatives were approx. 2. Separation between bronchodilating and depressor actions were also evident in I, II and AQ-110: the activity ratios of these compounds were 2-3 times that of Iso. On the other hand, III and IV had no significant selectivity on bronchodilating and depressor actions as compared with Iso.

Intraduodenal study

Table 2 represents the bronchodilating activities of Iso and the TMI derivatives, administered into the duodenum in anesthetized cats. As shown in Table 2, doses required for reducing the 5-HT-induced bronchoconstriction by approx. 70% of control were 100, 20, 20, 10, 30, 100 μg/kg for Iso, AQ-110, I, II, III and IV, respectively. Thus, II exhibited the most potent bronchodilating action, the potency of which was approx. 10 times that of

| Table 2. Bronchodilating actions of isoproterenol (Iso) and the TMI derivatives after intraduodenal administration and the ratio between intraduodenal and intravenous doses in anesthetized cats |
|-----------------|-----------------|-----------------|-----------------|
|                 | Intraduodenal   | Intravenous     | Intraduodenal   |
|                 | Dose (μg/kg)    | Peak Response  | Half-          | Dose (μg/kg) |  |
|                 |                 | % ± S.E.        | duration (min) |              |  |
| Iso             | 5               | 100             | 84.9 ± 6.0     | 75           | 0.20                      | 500 |
| AQ-110          | 6,7-OH         | 5               | 20             | 77.6 ± 5.2   | 150                      | 0.26 | 77 |
| I               | 7-OH           | 6               | 20             | 68.7 ± 7.7   | 210                      | 3.7  | 5  |
| II              | 5,7-OH         | 6               | 10             | 72.5 ± 6.1   | 210                      | 0.33 | 30 |
| III             | 5,6,7-OH       | 5               | 30             | 63.3 ± 6.7   | 120                      | 0.40 | 75 |
| IV              | 6,7,8-OH       | 5               | 100            | 68.0 ± 4.2   | 120                      | 1.3  | 77 |

a) Half-duration was defined as the period from the onset to the point of half recovery from its peak response. b) Dose which reduced the 5-HT-induced bronchoconstriction to the same extent in intraduodenal administration was calculated from dose-response curves in Fig. 2-A.
Iso. It was also found that time required for the peak activity was 15 min for Iso, 30 min for AQ-110, III and IV, 45 min for I and 60 min for II. On the other hand, the bronchodilating effects of I and II were considerably long-lasting and their half-durations were more than 210 min (Table 2). The half-durations for AQ-110, III and IV were 120–150 min, while that for Iso was 75 min. The maximum increase in heart rate, measured simultaneously with the bronchodilating action, were 57.6±8.3, 35.6±7.7, 23.2±2.8, 22.2±6.5, 24.8±5.1 and 26.2±3.4 beats/min for Iso, AQ-110, I, II, III and IV, respectively. Thus, when administered into the duodenum, the positive chronotropic actions of I, II, III and IV were weaker than those of AQ-110 and Iso.

For comparison of bronchodilating activities between intraduodenal and intravenous administrations of the test compounds, the i.d./i.v. dose ratio was calculated and is listed in Table 2. Doses were chosen which reduced the 5-HT-induced bronchoconstriction to the same extent in both routes of administrations. The i.d./i.v. dose ratios for I and II were 5 and 30 respectively, while those for AQ-110, III and IV were approx. 70. Iso produced the largest i.d./i.v. dose ratio among the compounds tested and such was approx. 500.

**DISCUSSION**

The intravenous administration of (+)-1-(3,4,5-trimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline (TMI) derivatives reduced the bronchoconstriction induced by 5-HT in anesthetized cats. Among five TMI derivatives, AQ-110 (6,7-dihydroxy analogue) exhibited the most potent bronchodilating activity, followed by those of 5,7-dihydroxyl (II), 5,6,7-trihydroxyl (III), 6,7,8-trihydroxyl (IV) and 7-hydroxyl (I) analogues in the decreasing order: the potencies of AQ-110, II, III, IV and I were 1/2.6, 1/4.0, 1/7.7, 1/23 and 1/36 that of Iso, respectively. Thus it was evident that the bronchodilating activities of the TMI derivatives were in order of dihydroxy-TMI > trihydroxy-TMI > monohydroxy-TMI among the compounds tested.

As shown in the present experiments, bronchodilating actions as well as positive chronotropic and depressor effects of the TMI derivatives were all inhibited by pretreatment with propranolol, suggesting that these compounds were acting as β-sympathomimetics. Iwasawa and Kiyomoto (1) have postulated that two hydroxyl groups at position 6 and 7 were necessary for full manifestation of β-sympathomimetic action of the TMI derivatives. The present results, however, indicate that hydroxyl group at position 6 is not necessarily required, since 7-hydroxyl and 5,7-dihydroxyl analogues also exhibited the β-sympathomimetic activity, although their potencies were lower than that of 6,7-dihydroxyl analogue.

It is worthy of notice that the TMI derivatives, as compared to Iso, exerted their actions more selectively on bronchial smooth muscle than on heart rate. This selectivity was most marked for I among the TMI derivatives tested. Therefore, it is inferred that the TMI derivatives are classified as β2-sympathomimetics (11, 12). On the other hand, the dose-response curve for I on either bronchodilating or positive chronotropic action was not parallel to those of the other TMI derivatives and Iso. Thus, as compared to the other TMI derivatives, different pharmacological mechanisms may be involved in the action of I.
It is of interest that the bronchodilating activities of I, II and AQ-110 were 2-3 times as potent as their depressor activities and these actions can be differentiated.

It has been reported that the bronchodilating activity of phenylethanolamine derivatives is reduced to 1/10-1/100 when hydroxyl groups of the catechol type were transformed to the resorcinol type, as represented by orciprenaline or terbutaline (13-17). In the case of TMI derivatives, however, the transformation from catechol type (AQ-110) to resorcinol type (II) did not cause any significant decrease in bronchodilating activity. On the other hand, it has been shown that the duration of the effect of resorcinol derivatives was longer than that of catechol derivatives (14, 15) and that the former compounds were less sensitive to catechol-O-methyltransferase (COMT) than the latter (18). The bronchodilating action of I (monohydroxy analogue) and II (resorcinol type) was also longer-lasting, suggesting that these compounds are little metabolized by COMT.

When administered into the duodenum, I and II produced a significantly longer bronchodilating action. In addition, the i.d./i.v. dose ratios of these compounds were smaller than those of the other TMI derivatives. These results suggest that I and II exhibit a greater absorption efficiency or intraduodenal bioavailability than the other compounds tested.

Acknowledgements: We thank professor Emeritus H. Kumagai, University of Tokyo, and Dr. K. Abe, Director of Biological and Chemical Research Laboratories, Tanabe Seiyaku Co., Ltd., for continued interest and support, and Dr. T. Nagao for pertinent advice and suggestions.

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